AMENDMENTS TO THE CLAIMS:

The listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-2 (cancelled).

Claim 3 (previously presented): A method of domain specific gene evolution comprising:

a) contacting a target nucleic acid encoding a polypeptide of interest with a recombinase and a first pair of single stranded-targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said nucleic acid encoding a first domain of said polypeptide to form a first recombination intermediate;

b) contacting said recombination intermediate with a single strand-specific nuclease to form a nicked target nucleic acid; and

c) reassembling and recombining said nicked or target nucleic acid to evolve a first library of altered target nucleic acids whereby said first predetermined sequence undergoes domain specific gene evolution.

Claim 4 (currently amended): A method according to claim 3 further comprising:

d) contacting said target nucleic acid or said first library of altered target nucleic acids with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide <u>of said second pair</u> comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

Claims 5-9 (cancelled)

Claim 10 (currently amended): A method according to any one of claims ~~1, 2,~~ 3, 4, ~~7, 8, 25,~~ 26, 30, 31, 32 and 33 ~~and 28~~ further comprising introducing the resultant product into cells to form a cellular library comprising variant nucleic acid sequences.

Claim 11 (previously presented): A method according to claim 10 further comprising expressing said cellular library of altered target nucleic acids to generate a library of variant polypeptides.

Claims 12 and 13 (cancelled).

Claim 14 (previously presented): A method according to claim 11 further comprising secreting said cellular library of variant polypeptides.

Claim 15 (cancelled)

Claim 16 (previously presented): A method according to claim 29 wherein said cell is eukaryotic.

Claim 17 (previously presented): A method according to claim 29 wherein said cell is procaryotic.

Claims 18-25 (cancelled).

Claim 26 (currently amended): A method according to claim 3 further comprising:

d) contacting said first recombination intermediate with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide of said second pair comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

Claims 27-28 (cancelled)

Claim 29 (currently amended): A method according to claim 3, 4, or 26 further comprising contacting said first recombination intermediate or said second recombination intermediate with a recombination proficient cell.

Claim 30 (new): A method of domain specific gene evolution of a target nucleic acid encoding a polypeptide sequence of interest, said method comprising:

contacting the target nucleic acid with a recombinase and a first plurality of pairs of single-stranded-targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid encoding a first domain of a polypeptide, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence, to form a first library of altered target nucleic acids;

repeating said contacting on said library of altered nucleic acids whereby said first predetermined sequence undergoes domain specific gene evolution; and

contacting said target nucleic acid or said first library of altered target nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each targeting polynucleotide of said second plurality of pairs comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids whereby said second predetermined sequence undergoes domain specific gene evolution.

Claim 31 (new): A method of generating a library of altered nucleic acids of a preselected target nucleic acid in a chromosomal sequence, said method comprising:

a) contacting a chromosomal nucleic acid comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting

polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to form a first library of altered target nucleic acids;

b) repeating step a) on said library of altered target nucleic acids; and

c) contacting said chromosomal nucleic acid or said first library of altered target nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide of said second plurality of pairs comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids, wherein said repeating is on said second library of altered target nucleic acids.

Claim 32 (new): A method of domain specific gene evolution of a target nucleic acid encoding a polypeptide sequence of interest, said method comprising:

contacting the target nucleic acid with a recombinase and a first plurality of pairs of single-stranded-targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid encoding a first domain of a polypeptide, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence, to form a first library of altered target nucleic acids;

repeating said contacting on said library of altered nucleic acids whereby said first predetermined sequence undergoes domain specific gene evolution; and

contacting all or part of said first library of altered nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially

complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide of said second plurality of pairs comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

Claim 33 (new): A method of generating a library of altered nucleic acids of a preselected target nucleic acid in a chromosomal sequence, said method comprising:

a) contacting a chromosomal nucleic acid comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to form a first library of altered target nucleic acids;

b) repeating step a) on said library of altered target nucleic acids; and

c) contacting all or part of said first library of altered target nucleic acids with at least one recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide of said second plurality comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids, wherein said repeating is on said second library of altered target nucleic acids.

Claim 34 (new): A method of domain specific gene evolution of a target nucleic acid encoding a polypeptide sequence of interest, said method comprising:

contacting the target nucleic acid with a recombinase and a first plurality of pairs of single-stranded-targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid encoding a first domain of a polypeptide, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence, to form a first library of altered target nucleic acids;

repeating said contacting on said library of altered nucleic acids whereby said first predetermined sequence undergoes domain specific gene evolution;

introducing the resultant product into cells to form a cellular library comprising variant nucleic acid sequences;

expressing said cellular library of altered target nucleic acids to generate a library of variant polypeptides; and

secreting said cellular library of variant polypeptides.

Claim 35 (new): A method of generating a library of altered nucleic acids of a pre-selected target nucleic acid in a chromosomal sequence, said method comprising:

a) contacting a chromosomal nucleic acid comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to form a first library of altered target nucleic acids;

b) repeating step a) on said library of altered target nucleic acids;

c) introducing the resultant product into cells to form a cellular library comprising variant nucleic acid sequences;

d) expressing said cellular library of altered target nucleic acids to generate a library of variant polypeptides; and

e) secreting said cellular library of variant polypeptides.